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A Second Function of the *S* Gene of Bacteriophage Lambda

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Infection of *Escherichia coli* by bacteriophage λ caused an immediate inhibition of uptake by members of all three classes of *E. coli* active transport systems and made the inner membrane permeable to sucrose and glycine; however, infection stimulated α-methyl glucoside uptake. Phage infection caused a dramatic drop in the ATP pool of the cell, but the membrane did not become permeable to nucleotides. Infection by only one phage per cell was sufficient to cause transport inhibition. However, adsorption of phage to the λ receptor did not cause transport inhibition; DNA injection was required. The inhibition of transport caused by λ phage infection was transient, and by 20 min after infection, transport had returned to its initial level. The recovery of transport activity appeared to require a λ structural protein with a molecular weight of 5,500. This protein was present in wild-type phage and at a reduced level in *S7* mutant phage but was missing in *S2* and *S4* mutant phage. Cells infected with *S7* phage had a partial recovery of active transport, whereas cells infected with *S2* or *S4* phage did not recover active transport. Neither the inhibition of transport caused by phage infection nor its recovery were affected by the protein synthesis inhibitors chloramphenicol and rifampin.

Infection of *Escherichia coli* by a number of different bacteriophage has been shown to alter the properties of the inner membrane (10). Puck and Lee (14) first suggested that the permeability of the inner membrane was drastically altered after infection by all T phages; however, more recent work has suggested that the major alterations seen by these authors are not caused by phage infection but instead are caused by lysis from without (8). There is strong evidence that the *E. coli* inner membrane becomes transiently permeable to potassium ions immediately after T4 phage infection (17, 18). A transient inhibition of the membrane potential has been shown to occur after infection of *E. coli* by both T4 and T5 phage (9). Adsorption of T4 phage ghosts to *E. coli* causes a permanent inhibition of potassium transport, inhibits a number of other transport systems, and causes the leakage of the acid-soluble phosphate pool (3). The latter two changes are not seen after infection by T4 phage. The cause of the changes in membrane properties observed after infection by T4 phage or T4 ghosts is not known.

This paper shows that λ infection caused an immediate increase in the permeability of the *E. coli* inner membrane. The permeability of the inner membrane returned to its normal level, even in the absence of protein synthesis, by 20 min after infection. The restoration of normal membrane permeability did not occur with certain λ *S* mutant phage, suggesting that a product of the λ *S* gene may be required for the restoration of permeability.

The *S* gene is a late gene of bacteriophage λ which is required for cell lysis (4). Recent work has suggested that a product of the *S* gene initiates cell lysis 40 min after infection by greatly increasing the permeability of the *E. coli* membrane (21).

MATERIALS AND METHODS

E. coli strain CSH7 (*lac* Str^r *thi*) was used as a nonpermissive strain and QD5003 (*su*$^+$) was used as a permissive strain for phage mutants. Strains WA321 (*thi met lac supE44 hsdR hsdM*) and WA2127 (*pel thi met lac supE44 hsdR hsdM*) were obtained from W. Arber, Biozentrum, Basel. Strain WA321 is the parent of WA2127. The λ phage used were λ *c*I857, λ *c*I857 *S7*, λ *c*I857 *S2*, λ *c*I857 *S4* and λ *c*I857 *S*ts. The λ *S2* and λ *S4* phage were obtained from E. Signer, Massachusetts Institute of Technology, Cambridge, and the other phage were obtained from J. Roberts, Cornell University, Ithaca, N.Y. Phage stocks were prepared by the shake dilute method (13) and had titers of 1×10^{10} to 3×10^{10} phage per ml.

Medium. T broth contained 10 g of peptone 20 (GIBCO Diagnostics), 5 g of sodium chloride, 2 g of maltose, and 2 g of magnesium chloride per liter.

Phage infection. Cells of the indicated strain were grown to 3×10^8 cells per ml in T broth at 37°C, and the indicated phage were added to give five phage per cell. The culture was shaken slowly at 37°C for 10 min to allow phage adsorption, and then the culture was shaken vigorously for the rest of the experiment.

Transport assays. Cells were harvested by centrifugation, and transport was measured by a membrane filter (Millipore Corp.) assay using 20- and 40-s time points as described previously (19). Fructose was used as a carbon source for proline and glutamine transport, whereas no carbon source was added for fructose and α-methyl glucoside transport. The substrate concentrations used in the transport assays were 8.3×10^{-6}, 1.66×10^{-5}, and 1×10^{-5} M for proline and glutamine, fructose, and α-methyl glucoside, respectively. The specific activities of the transport substrates were 10 μC/μmol, except for fructose, for which the specific activity was 2 μC/μmol.

Spheroplast stabilization. Uninfected and λ *S*2-infected cells were harvested by centrifugation at room temperature at an optical density at 600 nm of 0.4. The cells were washed by centrifugation with 0.01 M Tris (pH 8.0), and 2-ml samples were resuspended in 0.01 M Tris (pH 8.0) containing the indicated compound at a concentration of 20% (wt/vol). The optical density at 600 nm was measured, and then lysozyme and EDTA were added to give final concentrations of 0.1 mg/ml and 0.01 M, respectively. The samples were incubated at room temperature for 50 min, and the optical density at 600 nm was recorded for each sample. The polyethylene glycol used had an average molecular weight of 6,000.

Nucleotide release. CSH7 cells (10 ml) were grown at 37°C to an optical density at 600 nm of 0.15 in tryptone broth containing 0.2% maltose and 10^{-2} M magnesium chloride. Then potassium phosphate buffer (pH 7.5) was added to give a final phosphate concentration of 10^{-3} M along with 100 μCi of $^{32}P_i$, and growth was continued until the optical density at 600 nm reached 0.3. A sample of 5.0 ml was added to sufficient λ *S*2 phage in a separate flask to give five phage per cell. After 10 min, 1.0-ml samples from each culture were filtered through 0.45-μm (pore size) nitrocellulose filters. Each supernatant was collected in a flask which contained 1 drop of 70% formic acid. The filters and filtrates were treated as described previously (2). The final step in this procedure was chromatography on polyethyleneimine thin-layer plates to separate nucleotides. The dried thin-layer plate was exposed to X-ray film for 2 days.

RESULTS

The changes in proline transport resulting from the infection of cells of strain CSH7 by different λ phage are shown in Fig. 1. All of the phage caused greater than 95% inhibition of proline transport by 5 min after infection. When wild-type phage were used, transport returned to its original value by 20 min after infection. Transport returned to about 50% of its original value 20 min after infection by amber *S*7 or *S*ts mutant phage, whereas there was no recovery 20 min after infection by either *S*2 or *S*4 mutant phage. These results indicate that a product of the *S* gene is required for the reversal of the transport inhibition that is caused by phage infection. The inhibition of transport caused by phage infection did not result from harvesting and washing the infected cells, since inhibition still occurred when transport was measured directly on an infected culture (data not shown).

Neither the inhibition of transport nor its reversal required protein synthesis, since both processes occurred normally in cells in which protein synthesis was inhibited by chloramphenicol or rifampin (Fig. 2). This result indicates that the protein causing the reversal of transport inhibition does not have to be synthesized after infection and therefore must be present in the infecting phage.

To test this possibility, λ^+, λ *S*7, λ*S*2, and λ*S*4 phage, grown on strain CSH7 (*su*), were purified by banding in a cesium chloride density gradient (11). The purified phage proteins were separated by electrophoresis on a sodium dodecyl sulfate urea gel (Fig. 3). The λ^+ phage contained a protein with a molecular weight of 5,500 (determined from its mobility on the gel). This protein was missing in *S*2 and *S*4 phage and was present in reduced amounts in *S*7 phage. The presence of this protein in the phage correlated perfectly

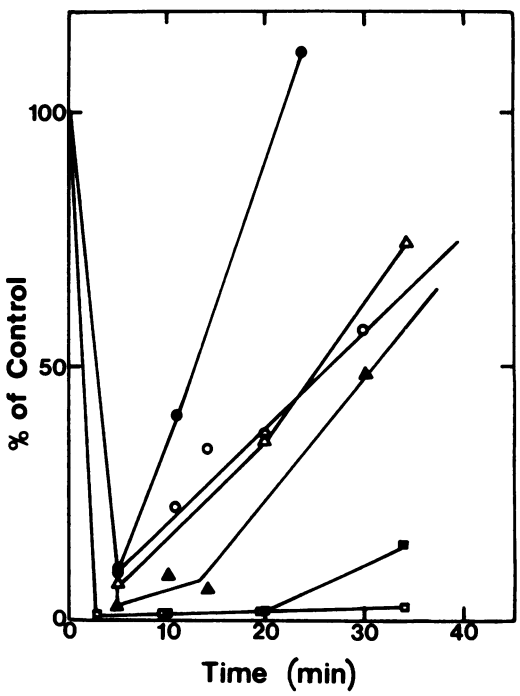

FIG. 1. Proline transport in cells infected with λ *S* mutant phage. Cells of strain CSH7 were grown in typtone broth containing 0.2% maltose and 10^{-2} M $MgCl_2$ to an optical density at 600 μm of 0.3. The indicated phage were added to give five phage per cell at time 0. Samples (2 ml) were taken at the indicated times and centrifuged, and proline transport was measured as described in the text. Uninfected cells, which were used as the 100% control, had a rate of proline transport of 4.3 nmol/min per mg of protein. Symbols: ●, λ^+; ○, λ *S*7 (*su*$^+$); ▲, λ *S*7 (*su*); △, λ R*S*ts; ■, λ *S*4; □, λ*S*2.

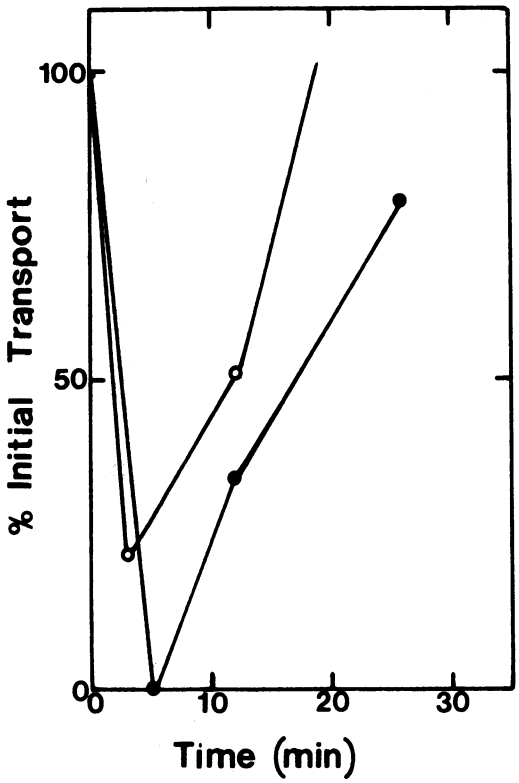

FIG. 2. Transport inhibition in the presence of inhibitors of protein synthesis. Cells of strain CSH7 were grown, infected, and assayed for proline transport as described in the legend to Fig. 1, except 400 μg of rifampin (○) or 30 μg of chloramphenicol (●) per ml were added just before phage infection.

with the degree of transport restoration that occurred after phage infection with the different mutant phage (Fig. 1). The 5,500-molecular-weight band was present at the same level in wild-type phage that had been rebanded in cesium chloride, indicating that it is a phage protein rather than a contaminant (data not shown).

The nature of the change resulting from λ infection was studied further by measuring transport by representatives of all three classes of *E. coli* transport systems (20) and by the α-methyl glucoside transport system, which is particularly resistant to most energy inhibitors (7). The results (Table 1) show that all of the transport systems were inhibited except for the α-methyl glucoside transport system, which was stimulated twofold. It is known that the α-methyl glucoside transport system is inhibited by the proton motive force (5, 15), and so the increase in uptake by this system indicates that λ infection dissipated the proton motive force.

Transport inhibition required λ DNA injection, since proline transport was not inhibited in λ-infected *pel* cells (Table 1). These cells adsorb λ phage but do not inject their DNA (16).

The inhibition of transport could result from an increase in the permeability of the *E. coli* inner membrane to small molecules, and an increase in permeability to sucrose and glycine was found. Sucrose and glycine were able to osmotically stabilize spheroplasts made from

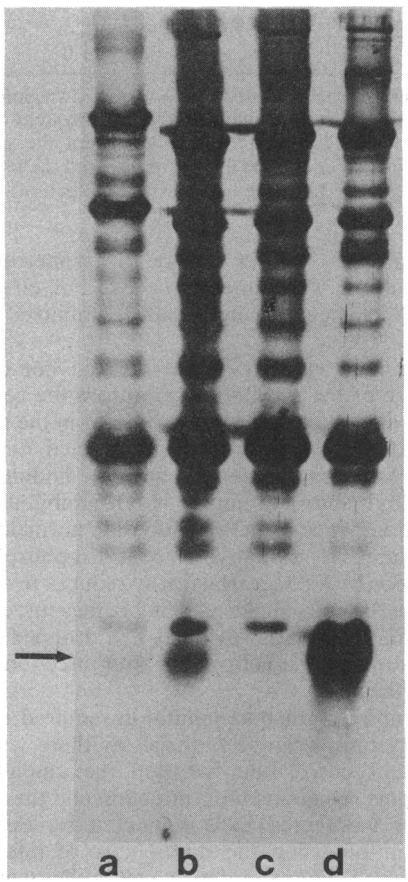

FIG. 3. Acrylamide gel electrophoresis of λ *S* mutant phage. Purified λ phage (16 μl of protein) were heated at 100°C for 3 min in a buffer containing 10 mM NaP_i (pH 7.2) 7 mM urea, 1% sodium dodecyl sulfate, 1% mercaptoethanol, and 0.01% bromophenol blue. The samples were loaded on a 15% acrylamide–0.4% bisacrylamide gel which contained 5.0 M urea, 0.1% sodium dodecyl sulfate, and 0.1 M Na P_i (pH 7.2) and electrophoresed for 18 h at 75 V. The running buffer contained 0.1 M Na P_i (pH 7.2) and 0.1% sodium dodecyl sulfate. The gel was stained with silver by a method described previously (12). The molecular weight of the *S* band was determined from a plot of log molecular weight versus mobility on the above gel, using as standards reduced insulin, the λ D, E, and L gene products, carbonic anhydrase, and ovalbumin. Lane a, λ *S2* phage; lane b, λ *S7* phage; lane c, λ *S4* phage; lane d, λ^+ phage.

TABLE 1. Effect of λ phage infection on *E. coli* transport activities

Strain	λ *S2*	Uptake (nmol/mg per min) of[a]:			
		Proline	Glutamine	Fructose	α-Methyl glucoside
CSH7	−	3.54	1.08	16.5	2.0
	+	0.21	0.37	1.5	4.3
WA321	−	0.60	0.76	22.4	0.7
	+	0.04	0.15	4.6	1.8
WA2127	−	1.2	1.4	16.4	1.5
	+	1.0	0.7	11.0	2.3

[a] Cells of the indicated strain were grown, infected, and assayed for the indicated uptake system as described in the legend to Fig. 1, except the growth medium also contained 0.2% fructose and the infected samples were harvested 15 min after infection.

control cells but did not stabilize spheroplasts made from λ *S2*-infected cells. Polyethylene glycol (molecular weight, 6,000) stabilized both types of cells.

The increase in permeability did not cause leakage of the nucleotide pool from the cell, as no labeled nucleotides were present in the medium after λ*S2* infection of ^{32}P-labeled *E. coli*. This result is in agreement with the finding that α-methyl glucoside uptake is not inhibited by λ infection, since α-methyl glucoside normally accumulates as α-methyl glucoside-6-phosphate. Infection by λ phage drastically reduces the ATP pool in the cell. The level of ATP, measured by a method described previously (1), dropped from 5.9 to 0.2 nmol/mg of protein after infection with λ *S2* phage.

Complete transport inhibition required infection by only a single λ phage, as there was an excellent correlation between the amount of transport remaining after infection and the number of uninfected cells predicted by Poisson distribution when the multiplicity of infection was varied. For example, at a multiplicity of 0.5, 66% of the control transport remained, and 60% of the cells should have received no phage.

DISCUSSION

The changes in *E. coli* cells resulting from λ infection indicate that infection caused a limited increase in the permeability of the inner membrane which made it permeable to glycine and sucrose but not to nucleotides or α-methyl glucoside phosphate. The increased permeability also dissipated the protein motive force and greatly reduced the ATP pool of the cell. Finally, all active transport systems tested except the α-methyl glucoside transport system were inhibited. The inhibition of the fructose transport system, which uses the same energy source, phosphoenol pyruvate, as the α-methyl glucoside uptake system is surprising but may be explained by the fact that the α-methyl glucoside uptake system has a significantly higher affinity for phospho heat-stable protein, the phosphate donor for the phosphotransferase transport systems, than does the fructose uptake system (M. Saier, personal communication).

The fact that the chain termination mutation present in the *S7* phage did not cause a complete loss of the 5,500-molecular-weight protein can be explained by the location of the *S7* mutation in the *S* gene. The *S7* mutation maps near the center of the gene (6), and the size of the chain termination fragment that would be produced by the *S7* mutation is predicted to be 5,670 from the DNA sequence of the *S7* mutant (D. Daniels and F. Blattner, personal communication). Thus, the protein present in *S7* phage could be either the chain termination fragment itself or, if the fragment is larger than the protein present in wild-type phage, the correct protein. The 5,500-molecular-weight protein may be present in reduced amounts in λ *S7* phage because processing of the termination fragment is less efficient than processing of the normal 11,000-molecular-weight product of the *S* gene, because the termination fragment is partially degraded, or because the chain termination fragment is not incorporated into λ phage as efficiently as the wild-type protein. The fact that the termination fragment produced by the *S7* mutant is large enough to give an active product also explains why *S7* phage grown on either Su^+ or Su^- cells gave almost identical resealing behavior (Fig. 1). The *S2* and *S4* mutations map near the N-terminal end of the *S* gene (6). The properties of these *S* mutants indicate that the 5,500-molecular-weight protein is probably the N-terminal fragment of the *S* gene product.

The results of this work show that the product of the λ *S* gene, which is predicted to code for a protein with a molecular weight of 11,000 by its DNA sequence (Daniels and Blattner, personal communication), is processed to produce a protein with a molecular weight of about 5,500 which is incorporated into native λ phage. This protein is required to repair the damage to the *E. coli* membrane which results from λ DNA injection. Work in this laboratory has shown that the λ *S* gene product is also processed, probably by both cross-linking and proteolysis, to form a 15,000-molecular-weight inner membrane protein which greatly increases the permeability of the *E. coli* inner membrane (A. Okabe and D. B. Wilson, submitted for publication). Therefore, the initial translation product of the λ *S* gene appears to be processed in two different ways to produce two proteins which both interact with

the *E. coli* inner membrane but have very different effects on the membrane.

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